Int.J.Curr.Microbiol.App.Sci (2017) *6*(3): 2414-2419

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 3 (2017) pp. 2414-2419**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.603.276

Insect Bioassay of *Beauveria bassiana* against Crawler Stage of Papaya Mealybug *Paracoccus marginatus* under Laboratory Condition

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Keywords

Paracoccus marginatus, *Beauveria bassiana*, Median lethal dose

Article Info

Accepted:
24 January 2017
Available Online:
10 March 2017

A B S T R A C T

Six strains of *Beauveria bassiana* were isolated from different sources collected from different places of Tamil Nadu and tested their efficacy under laboratory condition against crawler stages of papaya mealybug by insect dip bioassay method. The results revealed that *B. bassiana* strains, UPI (Bb) and ANR (Bb) were recorded the lowest LC_{50} values of 2.11 $x10^6$, 2.37$x10^7$ spores ml^{-1} respectively which were indicated more virulence compared to other strains. The LC_{50} values of rest of the strains of *B. bassiana viz.*, AVI (Bb), KPI (Bb), PLR (Bb), and TMR (Bb) were 6.81$x10^7$, 9.28 $x10^7$, 9.89$x10^7$ and 1.24$x10^8$ spores ml^{-1}. At the highest concentration of 1$x10^8$ spores ml^{-1}, the median LT_{50} values for different strains of *B. bassiana viz.*, UPI (Bb), ANR (Bb) , AVI (Bb), KPI (Bb), PLR (Bb) and TMR (Bb) were 3.71, 5.97, 7.72, 8.71, 9.25 and 9.71 days respectively. Median lethal values were found to be inversely proportional to the spore concentration of *B. bassiana*

Introduction

Papaya, *Carica papaya* Linn. (Family - Caricaceae), is an important fruit of tropical and sub-tropical region of the world (Katiyar *et al.*, 2008) and it is cultivated commercially as well as a backyard crop throughout the tropical world and in the warmest parts of subtropics. Papaya is affected by several arthropods among which Papaya mealybug is a polyphagous pest that can damage a large number of economically important field crops, tropical and sub-tropical fruits, vegetables and ornamental plants. Papaya mealybug infestations are typically observed as clusters of cotton-like masses on the above-ground portion of plants.

Colonization of mealy bugs on papaya has been noted along the veins and the midribs of the older leaves and all areas of tender leaves and fruits (Walker *et al.*, 2003). Severely affected older leaves turn yellow and dry up. Tender leaves become bunched and distorted. Heavy mealy bug populations produce a large volume of honey dew, which causes black sooty mould on the infested fruits and vegetation (Meyerdirk *et al.*, 2004). Entomopathogenic fungi are natural regulators of insect population and have potential as bio-pesticide agent against diverse insect pest in agriculture (Hall, 1984).

Materials and Methods

Survey, Isolation and purification of *B. bassiana* from naturally infected cadavers and soil

Survey was made during 2011 in different districts of Tamil Nadu on natural infestation to isolate and identify the fungi associated with naturally infected cadavers and soil samples table1.

Naturally infected cadavers showing outgrowth of fungi were collected and brought to laboratory for isolation and identification. *B. bassiana* was isolated from infected cadavers adopting the procedure of Lomer and Lomer (1995). The specimens were surface sterilized with 0.1 per cent sodium hypochlorite solution and rinsed with sterilized distilled water to remove the traces of sodium hypochlorite in order to prevent toxicity to the fungus. Sabouraud Dextrose Agar with yeast extract (SDAY) medium was used for isolation of the fungi and the slants were incubated in BOD incubator 25±2^{0}C and 80±10 RH until sporulation. *P. marginatus* crawlers and adults were inoculated with fungi and reisolated in pure form from the cadavers showing typical mycosis as per the procedure outlined by Goettel and Inglis (1997). The fungal species were got identified by experts of Indian Agricultural Research Institute, New Delhi. The isolated culture was maintained at 25±2^{0}C in a BOD incubator on SDAY. The pure stock culture was sub cultured at 15 day intervals in Petri plates (10 cm diameter). Pure stocks in slant were held under refrigerated condition until further use

For Isolation of *B. bassiana* fungi existing in the soil, it was done using the insect bait. Bait for this method were larvae of *Helicoverpa armigera*. Soil samples were collected from different regions of Tamil Nadu, brought back to the laboratory and kept in a refrigerator before use. Each soil sample was placed in plastic Petri dishes of 35mm in diametre and a small quantity of sterilized water was added to the dish. One to two larvae were placed in each dish and the dishes were kept at room temperature. These larvae were checked daily for mortality and dead ones were placed in 35 mm Petri dishes with moistened filter paper for sporulation (Shimazu, 1993).

To isolate the fungus, SDAY medium (Sabouraud Dextrose Agar supplemented with 1% of yeast extract) (barley flour 50 g; dextrose 10 g; neopeptone 4 g; yeast extract 2 g; agar 18 g; distilled water 1 L) were used. Conidia of the pathogenic fungi formed on the cadavers were taken by a mycological loop and streaked on SDAY medium. After incubation at room temperature 28±2ºC for a week, the colonies obtained were transferred to SDAY slant for preservation. The isolates were identified by microscopically inspecting the conidia forming mycelia for conidiogenous structure and conidial morphology (Samson *et al*., 1998)

Preparation and extraction of the spore of fungal strain

Mycelial discs of different isolates of *B. bassiana* were inoculated in SDY broth (Sabouraud's dextrose with 1% (w/v) yeast extract without agar) and incubated at 26°C for 48 hrs with shaking at 180 rpm. The flasks and plates showing luxuriant fungal growth were selected for harvesting spores and flooded with sterile distilled water containing 0.02 per cent surfactant, Tween 80 and Streptomycin sulphate 0.01 per cent. The spores were liberated by gentle agitation with silicone 'Policeman' and collected in sterile 250 ml Erlenmeyer flask. The final volume was made up to 100 ml with sterile distilled water. Subsequently, spore count was made with a haemocytometer. The spore concentration of the suspension was adjusted

to 10^8, 10^7, 10^6, 10^5 and 10^4 spores / ml with sterile distilled water by using serial dilution technique and they were used for bioassay against *P. marginatus*.

Insect Bioassay of *B. bassiana* in laboratory condition against crawler stage papaya mealybug

Papaya mealy bug populations were collected from the fields of papaya, tapioca belt and reared on sprouted potato at Department of Agricultural Entomology of TNAU, Coimbatore. Newly emerged crawlers were used for bioassay using standard insect dip method (Anonymus, 1990). Test solution was prepared by using sterile distilled water. The spore concentration of the suspension was adjusted to 10^8,10^7,10^6, 10^5 and 10^4 spores / ml with sterile distilled water by using serial dilution technique. Leaves with mealybugs were dipped in test solutions for one minute with gentle agitation and dried. After drying, they were placed in Petriplates containing fully set agar to avoid desiccation. Ten crawlers were used. Each treatment was replicated four times including control. The control leaves along with crawler were treated with sterile distilled water containing a 0.02% of Tween 80. All dishes were incubated at 25 ± 2^0C. Mortality was assessed after 3, 5, 7, 9 and 11 days after exposure of *B. bassiana*. The dead *P. marginatus*, which produced mycelial growth and failed to show movement after a gentle touch with a blunt lead pencil were considered for the mortality count (Hall 1984). Dead *P. marginatus* were collected and placed in petridish containing a moist filter paper and kept in humid chamber. The tested isolates were reisolated from the treated dead *P. marginatus* and were used for further experiments.

Statistical analysis

Mortality data was corrected with that in control by using the Abbot's formula (Abbott, 1925). The per cent corrected cumulative mortality of fungus as subjected to ANOVA test and the means were separated by LSD. The data was then analysed by probit analysis (Finney,1971) and the median lethal concentration and median lethal time values were computed by using statistical computer programme, Statistical Package of Social Science (SPSS) for all the strain were determined.

Results and Discussion

Median Lethal Concentration (LC_{50})

The results revealed that the significant difference was observed in concentration and time morality responses. The data given in the Table 2 indicate the LC_{50} values of six *B.bassiana* strains.

Table.1 Isolation of entomopathogenic fungi, *B. bassiana* from different places of Tamil Nadu

S.No	Name of the strains	Natural host	Locality	District	Date of isolates
1	AVI (Bb)	*Helicoverpa armigera*	Avinasi	Tiripur	08.11.11
2	ANR(Bb)	*Bombyx mori*	Annur	Coimbatore	17.11.11
3	KPI (Bb)	*H.armigera*	Kanchapalli	Coimbatore	22.11.11
4	UPI (Bb)	*B.mori*	Udumalaipet	Coimbatore	28.11.11
5	PLR (Bb)	*H.armigera*	Pongalore	Coimbatore	05.12.11
6	TMR(Bb)	Soil	Thondamuthur	Coimbatore	13.12.11

Table.2 Concentration - mortality responses of *P. marginatus* crawlers to different strains of *B. bassiana* by insect dip method

Isolate	Regression Equation	χ^2 P=0.05	LC_{50} (spore/ml)	Fiducial limits		LC_{95}	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
AVI (Bb)	Y=0.249x+3.034 R^2= 0.910	9.2481	$6.8153X10^7$	$2.2695X10^7$	$2.0949X10^8$	$6.6922X10^9$	$9.3556X10^8$	$4.7870X10^{10}$
ANR(Bb)	Y=0.249x+3.322 R^2= 0.953	11.1298	$2.3741x10^7$	$6.9267x10^6$	$8.1371x10^7$	$2.4433x10^9$	$4.5176x10^8$	$1.3215x10^{10}$
KPI (Bb)	Y=0.225x+3.150 R^2= 0.955	6.8326	$9.2876x10^7$	$2.8869x10^7$	$2.9879x10^8$	$1.1638x10^{10}$	$1.2103x10^9$	$1.1190x10^{11}$
UPI (Bb)	Y=0.269x+3.435 R^2= 0.998	11.3313	$2.1119x10^6$	$2.1879x10^6$	$3.7947x10^7$	$9.4751x10^8$	$2.2439x10^8$	$4.0008x10^9$
PLR (Bb)	Y=0.212x+3.243 R^2= 0.980	3.5159	$9.8972x10^7$	$2.9348x10^7$	$3.3342x10^8$	$1.4996x10^{10}$	$1.3029x10^9$	$1.7261x10^{11}$
TMR(Bb)	Y=0.225x+3.082 R^2= 0.974	4.4135	$1.2462X10^8$	$3.7976X10^7$	$4.0896X10^8$	$1.6462X10^{10}$	$1.4801X10^9$	$1.8310X10^{11}$

Bb- *Beauveria bassiana*, AVI (Bb)-Avinasi, ANR(Bb)- Annur, KPI (Bb)- Kanchapalli, UPI (Bb)- Udumalaipet, PLR (Bb)- Pongalore, TMR(Bb)- Thondamuthur

Table.3 Time - mortality responses of *P. marginatus* crawlers to different strains of *B. bassiana* at 1x108 spore/ml concentration by insect dip method

Isolate	Regression Equation	χ^2 (P=0.05)	LT_{50} (Days)	Fiducial limits		LC_{95} (Days)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
AVI (Bb)	Y=3.368x-4.727 R^2= 0.973	0.2793	7.72	5.87	11.47	27.56	9.30	81.69
ANR(Bb)	Y=3.576x-4.929 R^2= 0.984	0.3061	5.97	4.53	7.80	17.66	8.77	35.57
KPI (Bb)	Y=2.478x-2.289 R^2= 0.852	0.9318	8.71	5.06	22.06	75.49	4.02	1415.28
UPI (Bb)	Y=3.101x-2.969 R^2= 0.970	1.4842	3.71	3.00	12.21	20.67	3.65	116.94
PLR (Bb)	Y=2.632x-2.808 R^2= 0.971	0.3979	9.25	5.10	28.15	85.45	3.95	1848.15
TMR(Bb)	Y=2.741x-3.190 R^2= 0.922	0.3753	9.71	5.57	27.19	72.07	5.42	957.19

Bb- *Beauveria bassiana*, AVI (Bb)-Avinasi, ANR(Bb)- Annur, KPI (Bb)- Kanchapalli, UPI (Bb)- Udumalaipet, PLR (Bb)- Pongalore, TMR(Bb)- Thondamuthur

Among the six fungal *B. bassiana* strains, UPI (Bb) and ANR (Bb) caused 50 per cent mortality at lowest concentration of *B. basiana*, UPI (Bb) was found to be quite toxic with LC_{50} value of $2.11x10^6$ spore/ml followed by ANR (Bb) with LC_{50} value of $2.37x10^7$ spore/ml. AVI (Bb) and KPI (Bb) were found to be moderately toxic with LC_{50} values of $6.81x10^7$ and $9.28x10^7$ spore/ml respectively. PLR (Bb) and TMR (Bb) were

found to be less toxic (LC_{50} $9.89x10^7$ and $1.24x10^8$ spore/ml) as compared to other *B. basiana* strain respectively. It is understood that the lower LC_{50} values, higher will be the toxicity.

Median Lethal Time (LT_{50})

The LT_{50} values decreased with increased in concentration. At 10^8 spores ml^{-1}, The LT_{50} values of *B. basiana* strains *viz.,* UPI (Bb), ANR (Bb), AVI (Bb), KPI (Bb), PLR (Bb) and TMR (Bb) were 3.71, 5.97, 7.72, 8.71, 9.25 and 9.71 days respectively. Among *B. basiana* strains, UPI (Bb) was found to be causing 50 per cent mortality with LT_{50} value of 3.71 days followed by ANR (Bb) with LT_{50} value of 5.97 Days.

AVI (Bb) and KPI (Bb) were found to be moderately toxic with LT_{50} values of 7.72 and 8.71 days respectively. PLR (Bb) and TMR (Bb) were found to be slow active (LT_{50} 9.25 and 10.10 days) as compared to other *B. basiana* strains (Table 3). The lowest LC_{50} and The LT_{50} values of UPI (Bb) and ANR (Bb) indicate its higher virulence against crawler stage of *Paracoccus marginatus*. However other *B. basiana* strains also showed promising result against crawler. Tamai *et al.*, (1999) reported that *B. bassiana* could cause 50% mortality at concentration ranging from $5x10^6$ to$1x10^9$ spore/ml. Jeyarani *et al.*, (2011) also reported that *B. bassiana* could cause 50% mortality at 3.6 $x10^7$ spore/ml concentration is in conformity almost with present findings. Efficacy of *B. bassiana* against *P. marginatus* was also reported by Gulsar Banu *et al.* (2010). The difference in the LC_{50} values might be due to the difference in the virulence of fungal strains on the host species.

Acknowledgement

The authors very much thankful to staff, Division of plant pathology, Indian Agricultural Research Institute, New Delhi for their identification services.

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How to cite this article:

Indirakumar, K., J.S. Kennedy and Devi, M. 2017. Insect Bioassay of *Beauveria Bassiana* against Crawler Stage of Papaya Mealybug *Paracoccus marginatus* under Laboratory Condition. *Int.J.Curr.Microbiol.App.Sci.* 6(3): 2414-2419.
doi: https://doi.org/10.20546/ijcmas.2017.603.276